Aquaculture, 11 (1977) 355—362

© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

SOME EFFECTS OF ORAL DOSES OF OXYTETRACYCLINE ON GROWTH, SURVIVAL AND DISEASE IN *PENAEUS AZTECUS*

JANE CORLISS, DONALD LIGHTNER* and ZOULA P. ZEIN-ELDIN**

Southeast Fisheries Center, National Marine Fisheries Service, Miami, Fla. 33149,(U.S.A.)
**Environmental Research Laboratory, University of Arizona, Tucson International Airport, Tucson, Ariz. 85706 (U.S.A.)*
***Gulf Coastal Fisheries Center, National Marine Fisheries Center, National Marine Fisheries Service, Galveston, Texas 77550 (U.S.A.)*

(Received 5 April 1976; revised 6 May 1977)

ABSTRACT

Corliss, J., Lightner, D. and Zein-Eldin, Z.P., 1977. Some effects of oral doses of oxytetracycline on growth, survival and disease in *Penaeus aztecus*. *Aquaculture*, 11: 355—362.

Two size groups of brown shrimp (*Penaeus aztecus*) were fed a formulated feed containing 0, 100, 1 000 or 5 000 mg of oxytetracycline/kg of feed. Growth and survival were measured after a 3-week drug consumption period. The shrimp were then inoculated with *Vibrio alginolyticus*, and survival was monitored during the following 24 h.

At all three concentrations of oxytetracycline, small shrimp (mean initial wet weight 143.4 mg) consumed approximately one-third the amount of feed consumed by those fed the control diet with no oxytetracycline, yet growth was more rapid with diets containing 100 and 1 000 mg of oxytetracycline/kg of food than with the control diet. Larger shrimp (mean initial wet weight 458.1 mg) receiving oxytetracycline consumed about one-fourth the feed consumed by those on the oxytetracycline-free diet. Some growth inhibition was apparent in these shrimp at all oxytetracycline concentrations. Maximum drug consumption rate, based on actual feed intake, was approximately 1 300 mg oxytetracycline per kg body weight per day for small shrimp, but only 370 mg per kg body weight per day for larger shrimp.

All shrimp fed 0, 100 or 1 000 mg of oxytetracycline/kg of feed died within 24 h following inoculation with a standard dose (70% light transmission at 520—540 nm) of *Vibrio alginolyticus*. All the small shrimp and 70% of the large shrimp fed at the 5 000-mg drug level died, but death generally took place later in the 24-h period than with those fed at the lower drug concentrations. All shrimp fed 5 000 mg oxytetracycline/kg of feed and inoculated with a 1 : 100 dilution of the standard dose of *Vibrio alginolyticus* survived. All small shrimp and 90% of the large shrimp survived injection of sterile saline.

INTRODUCTION

Many bacterial infections in fish, including infections with *Vibrio* sp., have been successfully controlled with antibiotics (Bullock et al., 1971). Shrimp disease treatment has received little attention (Vanderzant et al., 1970, 1971;

Sindermann, 1971; Lightner and Lewis, 1975). Chan and Lawrence (1974) reported the effectiveness of oxytetracycline–oleandomycin combinations in reducing bacterial populations in larval shrimp cultures. They suggested that this antibiotic combination could be used to treat *Vibrio* and other bacterial infections in mysis and postlarval stage shrimp.

Two important points to consider before using a given drug are (1) the sensitivity of the organism causing the disease to the drug to be used, and (2) the effect of the drug on the diseased or healthy animal. In this paper, results of tests to evaluate oral administration of oxytetracycline to brown shrimp (*Penaeus aztecus*) are presented. Oxytetracycline was the antibiotic selected for initial experimentation for two reasons: (1) it has been cleared for use in farm animals and in fish by the U.S. Food and Drug Administration, and (2) it has been successfully used in treating certain fish diseases.

Concentrations of oxytetracycline used were based on those used successfully in the treatment of fish disease. The effects of the oxytetracycline were determined by comparing growth of treated and untreated control animals. It was assumed that detrimental physiological effects of the drug would be reflected in reduced growth of treated animals as compared to controls. To magnify possible negative effects of the drug, treated feed was given for 3 weeks, and two of the three concentrations were higher than dosages commonly used in fishes. The susceptibility of oxytetracycline-fed shrimp to *V. alginolyticus* infection was also tested. *V. alginolyticus* was classified and established sensitive to oxytetracycline through drug sensitivity tests by D.H. Lewis (personal communication, 1975). It has been shown to cause serious losses in cultured shrimp (Lightner and Lewis, 1975).

MATERIALS AND METHODS

Glass aquaria (30.5 × 75.0 × 30.5 cm) containing approximately 46 l of sea water were placed in rooms in which the temperature was maintained at 28 ± 2°C. The methods used for filtration and sterilization of the culture water are described by Zein-Eldin and Meyers (1973). Room design required that half of the aquaria be placed before fixtures fitted with two 40-W clear-white fluorescent bulbs (direct lighting), and half on a lower shelf receiving only indirect light from the above fixtures. Results from the two light intensities are reported separately. A light cycle of 12-h illumination and 12-h darkness was maintained.

Each aquarium was stocked with 20 brown shrimp from a single hatch, at the National Marine Fisheries Service Laboratory, Galveston, Texas. Initial mean total weight per tank was 2.87 g (143.4 mg mean weight) in Experiment 1 and 9.16 g (457.1 mg mean weight) in Experiment 2. Shrimp in duplicate tanks were fed diet 5-5/70B (Meyers and Zein-Eldin, 1973) containing, 0, 100, 1 000 or 5 000 mg of oxytetracycline/kg of food. The amount fed was increased or decreased daily, depending upon the amount eaten, so that shrimp always had food available. Growth and survival were determined after a 3-week

drug consumption period, and dead shrimp were removed daily.

Oxytetracycline consumption was expressed in milligrams of oxytetracycline per kilogram body weight per day for purposes of comparison with similar research for fishes. Because only final body weights were measured, calculation of approximate daily oxytetracycline consumption was made from the amount of food eaten during the last 3 days of testing. Approximate feeding rates, expressed as a percentage of body weight, were also calculated for the last 3 days. Conversion ratios were calculated as (total dry weight of food eaten/corrected total wet weight increase). In the manner of Kitabayashi et al. (1971), corrected total weights (W') were obtained as

$$W' = W + ((\bar{W}_i + \bar{W}_f)/2) \times N$$

where W = final total weight of the survivors,
$\bar{W}_i$ = average individual initial weight,
$\bar{W}_f$ = average individual final weight, and
N = number of shrimp dying in the experiment.

At the end of the 3-week period, ten shrimp from each oxytetracycline concentration were inoculated intramuscularly between the fifth and sixth abdominal segments with 0.02 ml of a suspension of *Vibrio alginolyticus* isolated and cultured in the pathology section of the laboratory (Lightner and Lewis, 1975). The cells were suspended in sterile saline (2% sodium chloride) and diluted to a standard concentration allowing 70% light transmission at a wave length of 520—540 nm (Perkin-Elmer spectrophotometer model 124-Coleman)*. There were approximately 10^7 cells/ml in this suspension. Cultures of the same strain of *V. alginolyticus* were used in both experiments. Ten shrimp (at least two individuals fed each drug concentration) were injected with a sterile saline solution in the same manner. In addition, the remaining shrimp from each antibiotic concentration in Experiment 1, but no more than ten, were given 0.02 ml of a 1 : 100 dilution of the *V. alginolyticus* suspension.

After inoculation, the shrimp were maintained for 24 h at 21—23°C. Dead shrimp were removed approximately 4, 8, 16 and 24 h following inoculations.

RESULTS

Addition of oxytetracycline to the feed decreased feed intake below that of the control regardless of the oxytetracycline level (Tables I and II). Feeding rates of the small, treated shrimp (Experiment 1) and of the large, treated shrimp (Experiment 2) were approximately one-third and one-fourth, respectively, that of their controls (Fig. 1).

Feed containing oxytetracycline was more efficiently converted (conversion ratios were lower) than feed without oxytetracycline. The controls in both

---

* Use of trade names in this publication does not imply endorsement of commercial products.

TABLE I

Results of the two replicates (A, B) of Experiment 1 after 3 weeks of feeding oxytetracycline at different concentrations. Initial sample weight of the shrimp in each replicate of the two trials was 2.87 g. Calculation of the corrected final total weight ($\Sigma W'$) is explained in the text

Mg oxytetracycline per kg of food	Final weight (g)		Corrected total weight ($\Sigma W'$) (g)		Corrected total weight increase (g)		Mean total weight increase ± SE (g)	Food consumed (g)		Conversion* ratio		Number dead		Total drug consumed (mg)	
	A	B	A	B	A	B		A	B	A	B	A	B	A	B
Direct light															
0	7.80	6.18	8.71	7.23	5.84	4.37	5.10 ± 0.51	66.9	53.0	11.5	12.0	3	4	—	—
100	9.04	8.48	10.46	9.82	7.59	6.95	7.27 ± 0.21	32.1	33.1	4.2	4.7	4	4	3.2	3.3
1 000	8.63	5.50	9.99	9.66	7.12	6.79	6.95 ± 0.09	30.0	28.0	4.2	4.1	4	11	30.0	28.0
5 000	4.94	5.79	8.13	8.30	5.26	5.43	5.34 ± 0.19	17.3	21.1	3.3	3.9	10	8	86.5	105.5
Indirect light															
0	5.97	5.85	7.68	7.03	4.82	5.06	4.94 ± 0.07	59.5	64.7	12.4	12.7	6	7	—	—
100	8.92	8.13	10.32	9.84	7.45	6.97	7.21 ± 0.15	35.0	29.6	4.7	4.2	4	5	3.5	3.0
1 000	5.70	8.24	8.18	9.97	5.31	7.10	6.20 ± 0.63	24.6	28.5	4.6	4.0	8	5	24.6	28.5
5 000	5.48	5.29	7.09	6.53	4.22	3.66	3.93 ± 0.19	18.7	18.8	4.4	5.1	6	5	94.5	94.0

* See text.

TABLE II

Results of the two replicates (A, B) of Experiment 2 after 3 weeks of feeding oxytetracycline at different concentrations

mg oxytetracycline per kg of food	Initial weight (g)		Final weight (g)		Corrected total weight ($\Sigma W'$) (g)		Corrected total weight increase (g)		Mean total weight increase ± SE (g)	Food consumed (g)		Conversion ratio		Number dead		Total drug consumed (mg)	
	A	B	A	B	A	B	A	B		A	B	A	B	A	B	A	B
Direct light																	
0	9.68	9.55	16.12	19.16	21.03	21.57	11.35	12.01	11.68 ± 0.21	117.0	147.0	10.4	12.2	6	3	—	—
100	7.99	9.21	14.20	13.34	14.20	17.58	6.21	8.37	7.28 ± 0.75	43.8	48.8	7.1	5.8	0	6	4.4	4.9
1 000	9.77	8.68	15.08	11.98	17.14	20.15	7.38	11.46	9.42 ± 1.43	40.8	45.6	5.5	4.0	3	10	40.8	45.6
5 000	8.75	10.00	3.89	8.34	12.97	14.00	4.22	4.01	4.11 ± 0.07	25.0	33.0	6.0	8.2	15	9	125.0	165.0
Indirect light																	
0	9.88	9.08	14.24	7.69	19.80	16.19	9.91	7.00	8.45 ± 1.02	102.0	157.0	10.3	22.4	7	12	—	—
100	9.31	8.14	4.32	7.38	12.61	13.10	3.30	4.97	4.13 ± 0.58	32.5	26.7	9.8	5.3	14	10	3.2	2.7
1 000	8.41	10.16	7.76	10.76	13.74	17.45	5.33	7.29	6.30 ± 0.68	27.2	38.0	5.1	5.2	10	9	27.2	38.0
5 000	9.32	8.67	8.10	6.16	14.47	11.41	5.15	2.74	3.94 ± 0.85	27.6	21.6	5.3	8.0	10	10	13.8	10.8

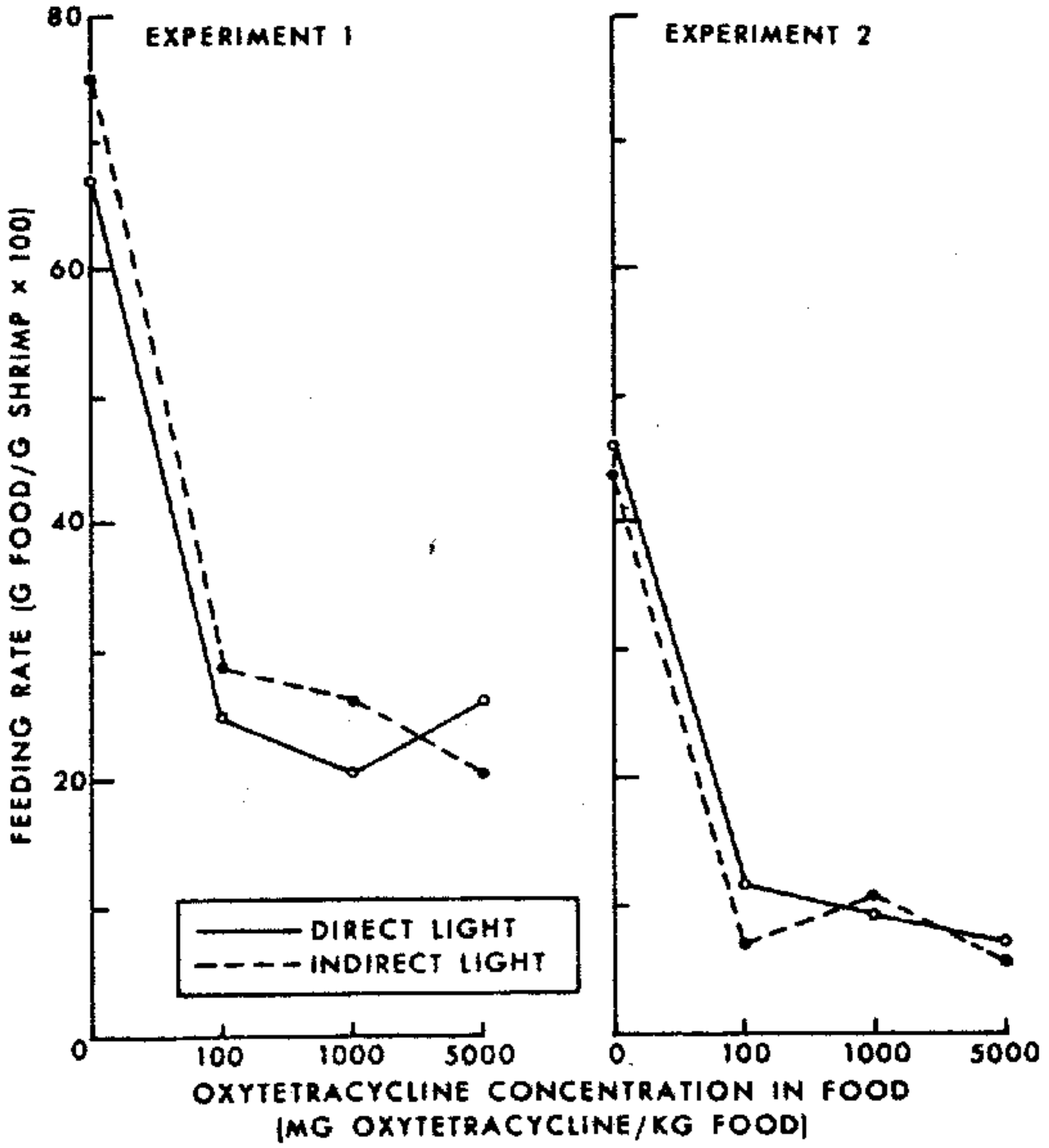

Fig. 1. Feeding rates ((grams of feed/grams of shrimp) × 100) of juvenile brown shrimp (*Penaeus aztecus*) fed oxytetracycline at different concentrations as determined during the last 3 days before final measurements. Experiment 1 — shrimp of mean initial weight 143.6 mg. Experiment 2 - - shrimp of mean initial weight 458.1 mg.

experiments had similar conversion ratios with the exception of one unexplained higher value.

The large shrimp showed less efficient conversion of feed containing oxytetracycline (conversion ratios were higher) than the small shrimp. The various oxytetracycline concentrations fed did not cause any appreciable change in feeding rate or conversion ratio.

Despite the decrease in feed intake, the small shrimp fed oxytetracycline at 100 and 1 000 mg/kg of food had growth rates significantly greater than those fed the control diet. At 5 000 mg/kg of food, weight increase of the small shrimp was not significantly different from that at 0 mg/kg of food. The large shrimp, however, showed a significant weight decrease at all drug concentrations, compared to their controls.

Survival was inconsistent both between duplicates and between experiments. Variation between experiments was probably related to variation in size of shrimp, differences in sample density, and the fact that the large shrimp had been held in captivity and fed an artificial diet longer than the small shrimp.

In Table III the effects of inoculation with *Vibrio alginolyticus* are presented. All control shrimp receiving a full strength inoculation died within 5 h of inoculation, and 80% of those given a 1 : 100 dilution died within 12 h of inoculation. Seventy percent of the large shrimp at the highest drug consumption level (360–387 mg drug/kg body weight) died within 20 h following full strength inoculation, while all the small shrimp at the highest drug level (1 295—1 316 mg oxytetracycline/kg body weight) died within 16 h. At other drug levels, shrimp of both size groups died within 10 h of inoculation. Survival at the highest drug concentration in both experiments was approximately 10 h longer than at the lower concentrations. Survival of the small shrimp was 100% with a 1 : 100 dilution at the highest drug concentration (1 295–1 316 mg drug/kg body weight).

Oxytetracycline was only effective in preventing *Vibrio alginolyticus* infections of limited severity (1 : 100 dilution) and then only at very high dosage levels. The lowest concentration of oxytetracycline to show any therapeutic effect (360 mg oxytetracycline per kg body weight per day) was greater than that necessary to treat infection in fish (Bullock et al., 1971). Since shrimp do not eat all the food immediately upon its being placed in the tank, leaching of the drug from the diet may be a possible explanation for the higher drug levels.

TABLE III

Number of shrimp dead 24 h after inoculation with *Vibrio alginolyticus* and number of shrimp inoculated

Experiment 1 — small shrimp (0.386—0.612 g)

Dilution of pathogen	Oxytetracycline concentration (mg drug per kg body weight per day)				
	0	22–27	180—230	1 295–1 316	Saline injected
1 : 0	10/10	10/10	10/10	10/10*	0/10
1 : 100	8/10	6/10	8/9	0/5	0/5

* Post-mortem appearance suggested 50% of the shrimp had survived inoculation for at least 16 h. At the other oxytetracycline concentrations, deaths apparently occurred within 6 h of inoculation.

Experiment 2 — large shrimp (0.710—1.198 g)

Dilution of pathogen	Oxytetracycline concentration (mg drug per kg body weight per day)				
	0	8—14	60—130	360—387	Saline injected
1 : 0	10/10	10/10	10/10	7/10**	1/10

** Animals also survived for a longer period of time than those receiving lower oxytetracycline concentrations.

In summary, mass mortalities have already been encountered in crustacean hatcheries and will undoubtedly occur again unless practical remedies are applied. To date, no effective therapy is available. Antibiotics that have been used successfully in treating fish diseases may be useful in crustacean aquaculture. During these experiments some growth stimulation was noted in the small shrimp from oral consumption of oxytetracycline, with the increase being greater at the two lower concentrations. There was an adverse effect on growth of the large shrimp at all oxytetracycline concentrations. At relatively high dosage levels some therapeutic value was evident. Further research is planned to establish a minimum prophylactic dosage level and to test the therapeutic value of oxytetracycline fed to previously infected shrimp.

REFERENCES

Bullock, G.L., Conroy, D.A. and Snieszko, S.F., 1971. Bacterial diseases of fishes. In: S.F. Snieszko and H.R. Axelrod (Editors), Diseases of Fishes, Book 2A. T.F.H. Publications, Jersey City, N.J., pp. 7–151.

Chan, E.S. and Lawrence, A.W., 1974. Effects of antibiotics on the respiration of brown shrimp larvae to postlarvae and bacterial populations associated with shrimp. In: J.W. Avault (Editor), Proc. 5th Annu. Workshop World Maricult. Soc. Louisiana State University, Baton Rouge, La., pp. 99—124.

Kitabayashi, K., Kurata, H., Shudo, K., Nakamura, K. and Ishikawa, S., 1971. Studies on formula feed for Kuruma prawn I. On the relationship among glucosamine, phosphorous and calcium. Bull. Tokai Reg. Fish. Res. Lab., 65: 91–108.

Lightner, D.V. and Lewis, D.H., 1975. A septicemic bacterial disease syndrome of penaeid shrimp. Mar. Fish. Rev., 37: 25–28.

Meyer, P., 1964. Field treatments of *Aeromonas liquefaciens* infections in golden shiners. Prog. Fish Cult., 26(1): 33–35.

Meyers, S.P. and Zein-Eldin, Z.P., 1973. Binder and pellet stability in development of crustacean diets. In: J.W. Avault and E. Boudreaux (Editors), Proc. 3rd Annu. Workshop World Maricult. Soc. Louisiana State University, Baton Rouge, La., pp. 351—364.

Sindermann, C.J., 1971. Disease-caused mortalities in mariculture, status and predictions. In: J.W. Avault, E. Boudreaux and E. Jaspers (Editors), Proc. 2nd Annu. Workshop World Maricult. Soc. Louisiana State University, Baton Rouge, La., pp. 69—74.

Sindermann, C.J. (Editor), 1974. Diagnosis and Control of Mariculture Diseases in the United States. Middle Atlantic Coastal Fisheries Center, Natl Mar. Fish. Serv., NOAA, U.S. Dep. Commer. Tech. Ser., Rep. No. 2, December. Highlands, N.J., 306 pp.

Vanderzant, C., Mroz, E. and Nickelson, R., 1970. Microbial flora of Gulf of Mexico and pond shrimp. J. Milk Food Technol., 33: 346–350.

Vanderzant, C., Nickelson, R. and Judkins, P.W., 1971. Microbial flora of pond-reared shrimp (*Penaeus aztecus*). Appl. Microbiol., 21: 916—921.

Zein-Eldin, Z.P. and Meyers, S.P., 1973. General consideration of problems in shrimp nutrition. In: J.W. Avault and E. Boudreaux (Editors), Proc. 4th Annu. Workshop World Maricult. Soc. Louisiana State University, Baton Rouge, La., pp. 299—317.